PATENT
603-1-009

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S): HENRY W. FOUNDS et al.

SERIAL NO.: 08/367.507 EXAMINER: Adams, D.

FILED: December 30, 1994 ART UNIT: 1816

FOR: MONOCLONAL ANTIBODY SPECIFIC FOR ADVANCED GLYCOSYLATION ENDPRODUCTS IN BIOLOGICAL SAMPLES

DECLARATION OF HENRY W. FOUNDS, PH.D
UNDER 37 C.F.R. § 1.132

CERTIFICATE OF MAILING UNDER 37 CFR 1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to the ASSISTANT COMMISSIONER OF PATENTS, WASHINGTON, DC 20231 on February 3, 1997.

Paul F. Fehlner, Ph.D. Reg. No. 35,135 2/3/97
(Name of Registered Representative) (Signature and Date)

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

HENRY W. FOUNDS, PH.D. hereby declares and states that:

1. I am a co-inventor of invention disclosed and claimed in the above-identified patent application.

2. I am presently employed by Alteon Inc. of Ramsey, New Jersey, assignee of the invention and related application, where I have been employed since 1992 as Director, Diagnostic Programs. In that capacity I have supervised the development of immunoassays for the detection of advanced glycation end-products (AGEs).

3. My qualifications are as follows:

(a) Summary

Undergraduate education in biology and chemistry with advanced education in microbiology. Advanced training in cell culture and virology was attained at the graduate level with training and applied experience in immunochemistry and immunoassay over a 9 year period in the industry.

(b) Work Experience

Ventrex Laboratories, Inc., Portland, Me. 1983-1992: various research and development positions from bench scientist to Senior Vice President, Research and Development. Directed the development of immunoassays for various blood analytes, allergy diagnostics, and biotechnology products for the immunology market. Developed the first rapid enzyme immunoassay for Streptococcus A, a diagnostic for Streptococcal sore throat. The products were sold through dealers to the hospital, clinical, and physicians laboratory market.

Personal Diagnostics, Inc., Parsippany, N.J., 1981-1983: consultant microbiologist. Participated in the early stage development of a rapid automated test for UTI.

(c) Education

B.S. in Biology, Villanova University
M.S. in Biology, University of Notre Dame
Ph.D. in Microbiology, Rutgers University

(d) Professional Memberships

American Association for Microbiology (ASM)
American Diabetes Association (ADA)
American Association for Clinical Chemistry (AACC)
Molecular Medicine Society (MMS)

(e) Patents and Publications

Patent No. 4,794,076 - December 27, 1988
Simultaneous Extraction of a Ligand from a Sample and Capture by Anti-Ligands, therefore, in Ligand/Anti-Ligand Assays.

Patent No. 4,668,632 - May 26, 1987
Sparger and Apparatus for and Method of Growing Cells

"Tobacco Smoke is a Source of Advanced Glycation Endproducts: Possible role in the accelerated vascular disease of smokers". *Journal of Investigative Medicine*, 1996; 44(3):200A.

Wolffenbuttel, B., Giordano, D., Founds, H., Bucala, R., Long-term assessment of glucose control by hemoglobin-AGE measurement, *The Lancet*, 1996; 347:313-315.

Ateshkadi, A., Johnson, C., Zimmerman, S., Serum Advanced Glycosylation End-Products in Patients on Hemodialisis and CAPD, *Portioneal Dialysis International*, 1995; 15:129-133.

"Significant Reduction of Plasma LDL-ApoB and Glycated ApoB follows Chronic High-Flux Hemodialysis in Diabetic Uremic Patients", 28th Annual Meeting, American Society of Nephrology, 1995.

"Kinetic Analysis of Fructose-Modified Protein Formation Using a New Immunoassay Method," American Diabetes Association, Las Vegas, Nevada, 1993.

"Step Saver - An Automated Allergy System." American in Vitro Allergy and Immunology Society, Vail, Colorado, 1991.

4. I have reviewed the Office Action dated July 31, 1996, in connection with the above-identified application, and the references cited therein. This Declaration was prepared

in order to demonstrate for the record that of all the claimed monoclonal antibodies are distinct from a monoclonal antibody as described by Horiuchi *et al.* [JBC **226**:7329-32 (1991)] (hereinafter "Horiuchi") and Araki *et al.* [JBC **267**:10211-14 (1992)] (hereinafter "Araki"), and the antibodies described by Bucala [WO 93/13421]. Accordingly, the following experiments were conducted by me or at my direction to evaluate the binding activity of the claimed monoclonal antibody to AGE-antigens.

5. **The claimed antibody is distinct from the monoclonal antibody described by Horiuchi/Araki.** The following experiment demonstrates that the claimed monoclonal antibody has clearly distinct antigen binding properties, and thus epitope specificity, compared to the monoclonal antibody described in Horiuchi/Araki, and that both of these references relate to the same monoclonal antibody.

(a) Materials and Methods

Competitive ELISA with monoclonal antibody 4G9. A competitive immunoassay as described in Horiuchi (page 7329-7330) was performed. 6-Aminocaproic acid (6ACA), 4-aminobutyric acid (4ABA), and β-alanine (βAla) were browned with glucose (-Glu) 90 days (6ACA and 4ABA) or 56 days (βAla) according to the Horiuchi protocol. This material was tested in the competitive ELISA format, and 50% inhibition values in μg/well obtained. Monoclonal antibody 4G9 is the antibody described in the instant application (page 6, lines 14-16).

Monoclonal antibody 6D. The data for monoclonal antibody 6D were obtained by extrapolation from Figure 3, right panel.

(b) Results

The 50% inhibition values in μg/well are given for 4G9 and compared with the published results for 6D (Horiuchi, Figure 3). These results are shown in the table, below:

Table: Monoclonal Antibody Specificity

AGE-antigen	50% Inhibition of Monoclonal Antibody	
	4G9	6D
6ACA-Glu	0.0074	2.9
4ABA-Glu	0.11	19.
βAla-Glu	1.1	16.

These results show that 4G9 has nearly 400-fold greater apparent affinity for 6-aminocaproic acid reacted with glucose than 6D. In addition, 4G9 has a clearly different reactivity pattern for 4-aminobutyric acid reacted with glucose and β-alanine reacted with glucose. There was no reactivity of 4G9 with unbrowned amino acids.

(c) These data clearly show that the claimed monoclonal antibody is distinct from the monoclonal antibody described by Horiuchi. Antibody 4G9 has much higher apparent affinity for one antigen, and a different reactivity profile. These data represent distinct epitope specificities of the claimed antibody and the Horiuchi antibody.

(d) Araki reports binding activity of the same antibody described by Horiuchi: "Polyclonal and monoclonal anti-AGE antibodies were prepared and characterized as reported

previously ([Horiuchi et al., *J. Biol. Chem.* **266**:7329-7332 (1991)])" (Araki, page 10212, top of column 1).

6. **Although one of the genus of antibodies described in Bucala, the claimed monoclonal has patentably distinct characteristics.** The following data (Figures 1-4; Appendix A) show that the claimed monoclonal antibody has surprising characteristics relative to the antibodies described in Bucala.

(a) **The claimed monoclonal antibody reacts with an apparent high affinity with carboxymethyllysine.** A competitive assay was performed with the claimed monoclonal antibody according to the protocol for competitive ELISA on page 26, lines 11-17 of the specification. Carboxymethyllysine was used as the inhibitor antigen. The data in Figure 1 attached hereto show that carboxymethyllysine inhibited 50% of binding of 4G9 to AGE-BSA at about $7x10^{-8}$ M, *i.e.*, about 70 nM. In contrast, Bucala reports very low reactivity with CML (see Bucala, Figure 2C, which shows inhibition of antibody binding only at very high levels of carboxymethyllysine).

(b) **The claimed monoclonal antibody shows relatively greater affinity for AGEs than polyclonal antibodies corresponding to the antibody described by Bucala.** A comparative thiocyanate elution affinity analysis according to Macdonald et al. [*J. Immunol. Methods* **106**:191-194 (1988)] was performed with the nine monoclonal antibodies produced by hybridomas from the group 5D2-4D6 (specification, page 24, lines 1-4). This comparative analysis included a polyclonal anti-KLH-AGE antibody (termed KLH2951; specification, page 21, line 20 to page 22, line 21), and an anti-RNase-AGE polyclonal antibody as described in

Bucala (Bucala, page 36, lines 33-35; page 39, line 22 to page 40, line 11). The results of this analysis show that monoclonal antibody 4G9 has higher relative affinity for BSA-AGE than either of the polyclonal antibodies.

(c) **The claimed monoclonal antibody has greater sensitivity for serum AGEs and AGEs in skin samples than the Bucala antibody.** AGE levels were detected in two different biological samples: diabetic human patient sera prepared and tested as set forth in the protocol attached in Appendix B; and Charles River Hairless mouse skin samples prepared according to the protocol set forth in the specification with respect to measurement of AGE levels in rat skin (specification, page 31, lines 4-34). The results are shown in Figure 3 and 4 respectively. Both figures demonstrate greater reactivity of the monoclonal antibody than the rabbit polyclonal anti-RNase-AGE antibody.

(d) Although the Bucala antibodies have the properties demonstrated by the claimed monoclonal, there is no way to predict which, if any, of these properties will predominate in a particular monoclonal, to what degree the monoclonal will evidence such properties, and what advantages these properties may confer. The teachings of Bucala did not lead and would not have led me to develop a monoclonal having the characteristics of the 4G9 monoclonal of this application, which has high specificity for carboxymethyllysine, greater affinity for BSA-AGE in a thiocyanate comparative affinity assay, and greater sensitivity for AGEs on serum peptides and in skin than the antibodies described by Bucala. Thus, although the claimed monoclonal is a species of Bucala antibody, it is in my scientific opinion a distinct species that merits individual protection.

7. In view of the foregoing data, the claimed monoclonal antibody is clearly distinct from the monoclonal antibody of Horiuchi/Araki, and has unexpected properties relative to the antibody of Bucala.

8. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 2/3/97

Henry W. Founds, Ph.D.
HENRY W. FOUNDS, PH.D.

APPENDIX A

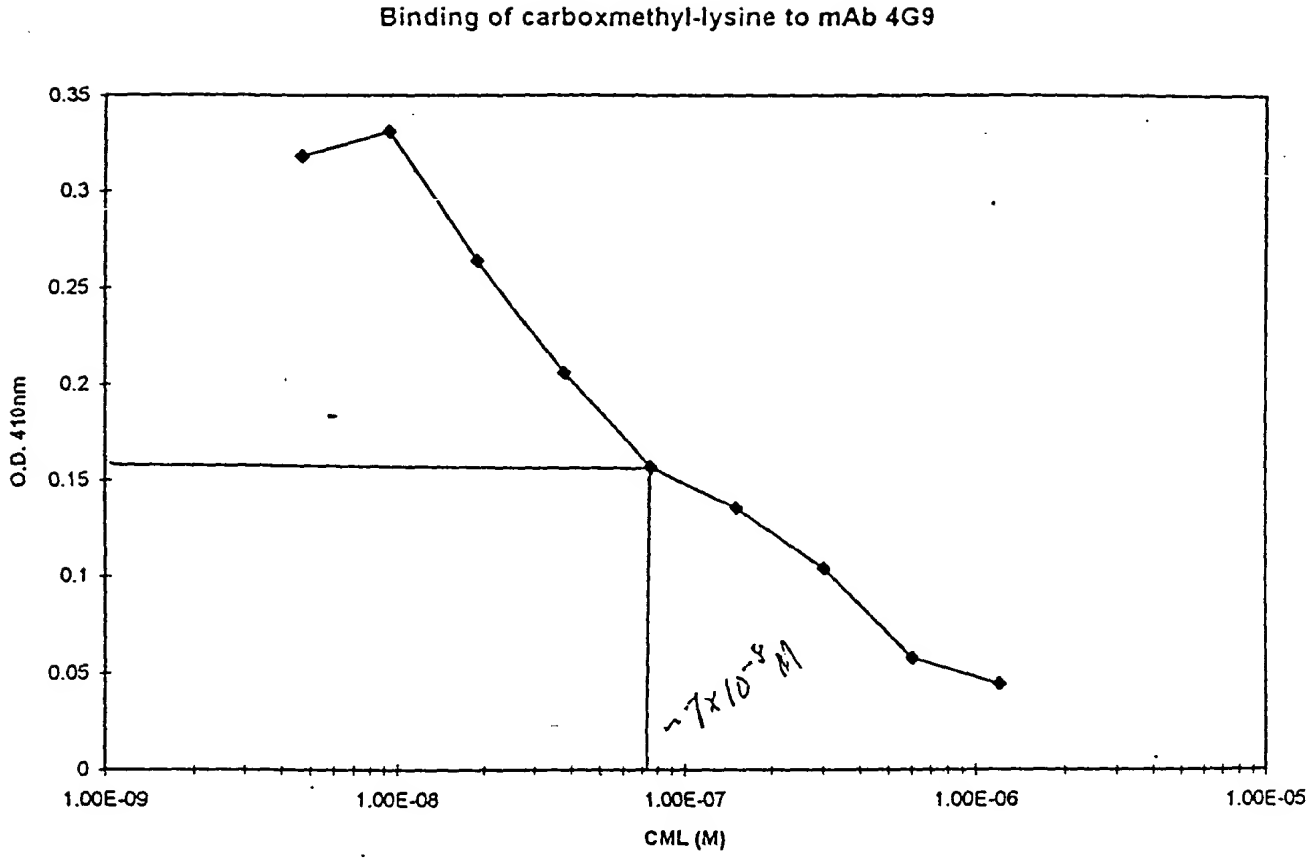

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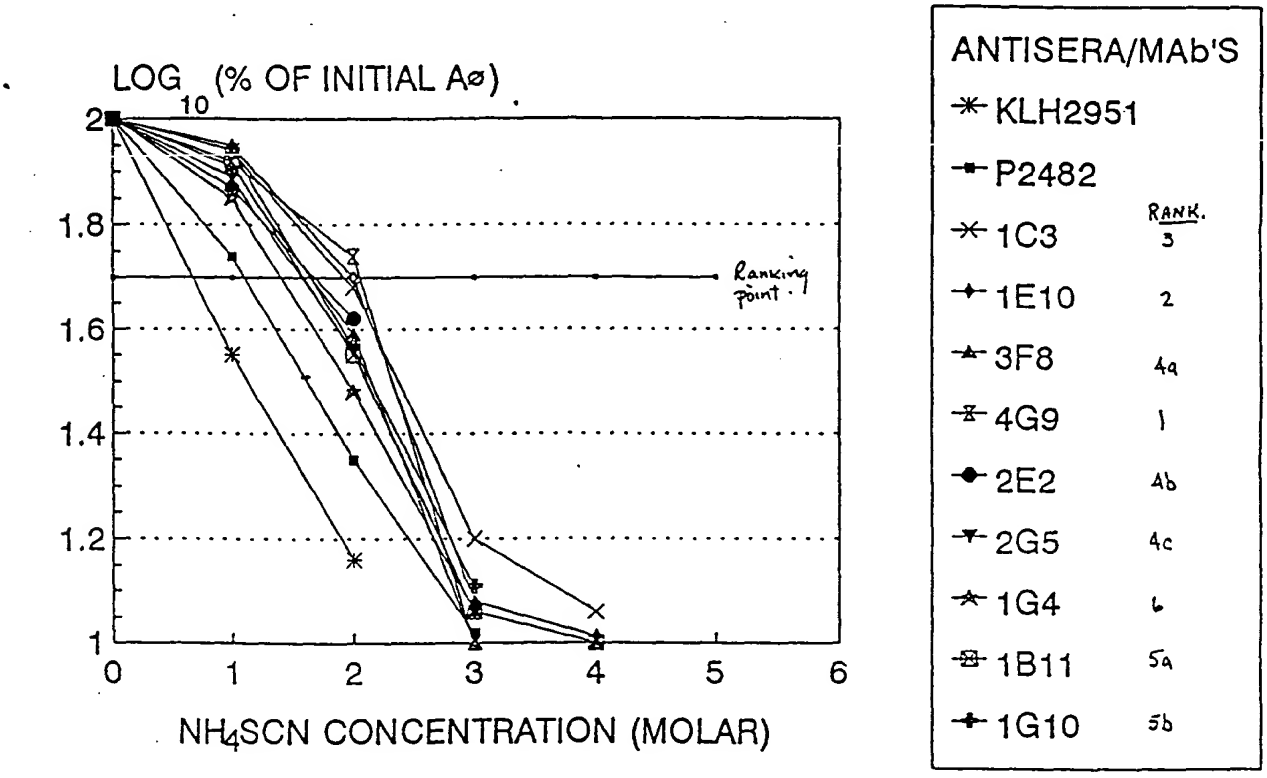
FIGURE 1

THIOCYANATE ELUTION AFFINITY ANALYSIS

POLYCLONAL ANTISERA AND SUBCLONE SUPERNATES OF 5D2-4D6 VS. BSA-AGE



ASSAY#PD1/4-GI3

FINAL DILUTION: MAb'S-1:4PBS/.02%TWEEN; P2482-1:1400PBSDB; KLH2951-1:10,000PBSDB

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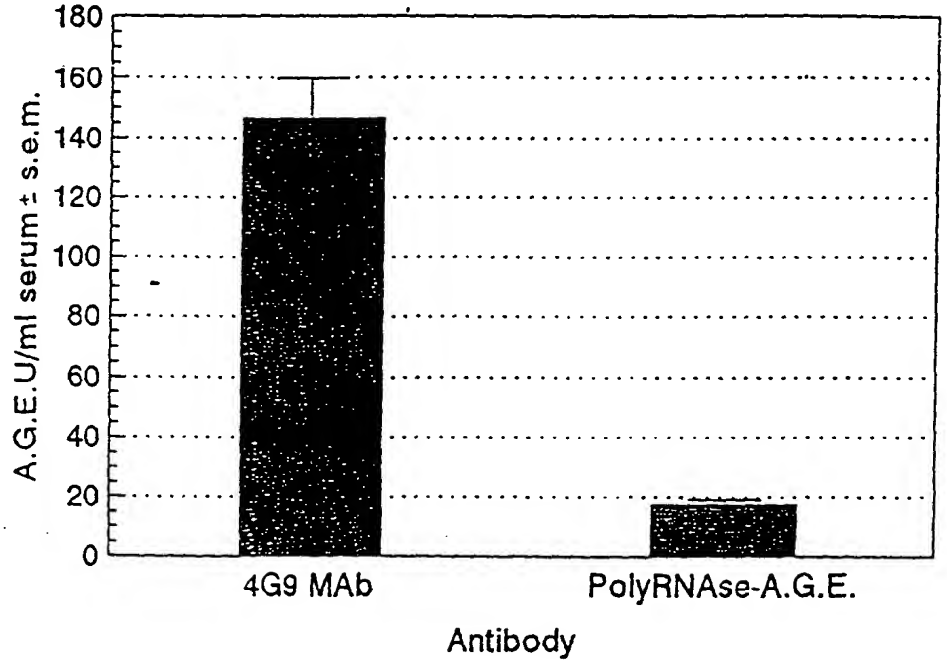
FIGURE 2

Serum-A.G.E. Analysis of 10 Diabetic Patients

Reactivity Comparison 4G9 Monoclonal vs. RNAse-A.G.E. Rabbit Polyclonal



Serum Digested with proteinase K/proteinase inhibitor added before analysis

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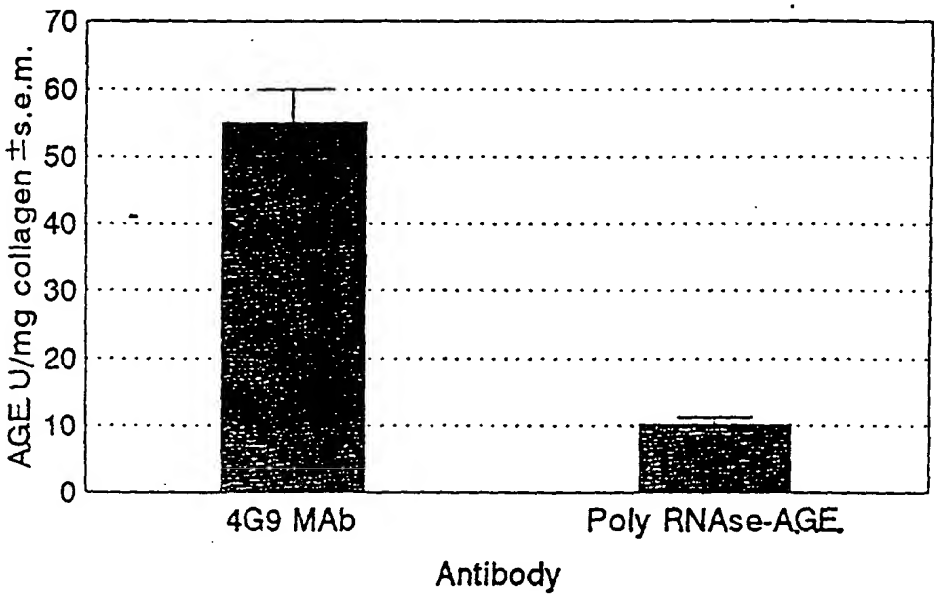
FIGURE 3

Charles River Hairless Mouse Model

A.G.E. Content of Collagenase Digest of Epidermis
Reactivity Comparison 4G9 Monoclonal vs. RNAse-AGE Rabbit Polyclonal



Skin digests passed through a 10K membrane and ultrafiltrate analyzed
n=10 animals

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FIGURE 4

APPENDIX B

Meth ds and Materials

Reagent solutions: 1)Enzyme Stock Solution: An 8mg/ml stock solution of Proteinase K (Gibco #25530-31) is made in 50mM Tris buffer, pH8.0 and stored at -20°C for up to 2 weeks. 2) Enzyme Working Solution(EWS): Dilute the Enzyme Stock Solution 1:20 in 0.02M Sodium Phosphate Buffer(SBPB), pH 7.4 containing 0.02% sodium azide. Phenylmethylsulfonyl Fluoride Solution (PMSFs) (Sigma #P-7626): Dissolve 0.174 g of PMSF in 10ml of reagent grade ethanol in a glass vial (100mM Stock PMSF). Mix to dissolve and make a 1:200 dilution of the PMSF Stock solution in SBPB (PMSFB).

Preparation of the Serum Sample and Negative Control: 1) Pipette 50 ul of serum into a microfuge tube and add 100ul of EWS, vortex to mix and place on a rocker in a 37°C incubator for 18 hours. 2) Negative Enzyme Control: add 50ul of SPB and 100ul of EWS into a microfuge tube and vortex. Follow the procedure as with the serum sample.

Final Preparation of the Sample and Negative Control: Remove the samples and negative enzyme control from the incubator and centrifuge for 10 minutes at 13,500 rpm in a Biofuge 15 (Baxter). Remove 100 ul of the supernatant sample (serum and negative control) and place in a fresh microfuge tube. Add 400 ml of PMSFB to the tube containing the 100ul of supernate and vortex to mix.

Competitive A.G.E. ELISA: 50ul of the prepared sample and negative control is added to the wells of a microtiter plate and the A.G.E. competitive ELISA procedure is followed as deseribed in the application. The sample has been diluted 1:15 by this procedure and is corrected for this dilution after analysis and expressed as A.G.E. units/ ml serum. The negative enzyme control is used as the %Bo against which the %Bn of the samples are normalized and read against the standard curve - BSA-AGE as described in the application.